

AMENDMENTS TO THE CLAIMS

1. **(Currently amended)** A method of high throughput quantification of a specific mRNA in whole blood, comprising the steps of:

- (a) collecting whole blood;
- (b) administering an anticoagulant to the whole blood;
- (c) removing erythrocytes and blood components other than leukocytes from the whole blood by filtration to yield leukocytes comprising eosinophils on a filter membrane;
- (d) lysing the leukocytes on a filter membrane to produce a lysate comprising mRNA including said specific mRNA;
- (e) transferring the lysate to an oligo(dT)-immobilized plate to capture the mRNA; and
- (f) quantifying the specific mRNA.

2. **(Cancelled)**

3. **(Original)** The method of Claim 1, wherein heparin is administered to the whole blood prior to collection of leukocytes.

4. **(Currently amended)** The method of Claim 1, wherein the whole blood is frozen and subsequently thawed prior to filtration.

5. **(Original)** The method of Claim 1, wherein the filter membrane is attached to a multi-well filter plate.

6. **(Currently amended)** The method of Claim 1, wherein the filter membrane is a PBT polybutylene terephthalate (PBT) fibrous membrane.

7. **(Original)** The method of Claim 5, wherein the leukocytes are captured on a plurality of filter membranes layered together.

8. **(Original)** The method of Claim 1, additionally comprising washing the leukocytes on the filter membrane with hypotonic buffer to further remove erythrocytes and other blood components.

9. **(Original)** The method of Claim 8, additionally comprising drying the filter membrane.

10. **(Original)** The method of Claim 9, wherein the filter membrane is washed with ethanol and subjected to vacuum aspiration until the filter membrane is dry.

11. **(Original)** The method of Claim 1, wherein the immobilized plate comprises a multi-well oligo(dT)-immobilized plate.
12. **(Original)** The method of Claim 1, wherein the transfer of lysate to the oligo(dT)-immobilized plate comprises centrifugation.
13. **(Withdrawn)** The method of Claim 1, wherein the transfer of lysate to the oligo(dT)-immobilized plate comprises vacuum aspiration.
14. **(Withdrawn)** The method of Claim 1, wherein the transfer of lysate to the oligo(dT)-immobilized plate comprises applying positive pressure.
15. **(Original)** The method of Claim 1, wherein the quantification of mRNA comprises cDNA synthesis of the specific mRNA and amplification of resulting cDNA.
16. **(Withdrawn)** The method of Claim 1, wherein the mRNA quantified is β -actin mRNA.
17. **(Withdrawn)** The method of Claim 1, wherein the mRNA quantified is CD4 mRNA.
18. **(Withdrawn)** The method of Claim 1, wherein the mRNA of a translocation gene involved in leukemia is quantified.
19. **(Withdrawn)** The method of Claim 1, wherein the mRNA of cancer-specific genes from micrometastatic cancer is quantified.
20. **(Withdrawn)** The method of Claim 1, wherein virus-derived mRNA from infected white blood cells is quantified.
21. **(Withdrawn)** The method of Claim 20, wherein the quantified virus-derived mRNA is HIV
22. **(Withdrawn)** The method of Claim 21, wherein the quantification of HIV mRNA is used to diagnose HIV.
23. **(Withdrawn)** The method of Claim 20, wherein the quantified virus-derived mRNA is CMV.
24. **(Withdrawn)** The method of Claim 23, wherein the quantification of virus-derived mRNA is used to diagnose CMV.
25. **(Withdrawn)** The method of Claim 20, wherein the quantification of virus-derived mRNA is used to monitor blood banks for the presence of viral diseases.

26. **(Withdrawn)** The method of Claim 20, wherein the quantification of virus-derived mRNA is used to study anti-viral drug sensitivity.

27. **(Currently amended)** The method of Claim 1, wherein the specific mRNA of is mRNA known to be induced during apoptosis genes involved development in leukemia is quantified.

28. **(Original)** The method of Claim 1, wherein the mRNA of cytokines is quantified.

29. **(Currently amended)** The method of Claim 1, wherein the quantification of mRNA is used to test the side effects of anti-cancer drugs on white blood cells that induce specific mRNA responsible for apoptosis development in leukocytes.

30. **(Withdrawn)** The method of Claim 1, wherein the mRNA of DNA-repair genes is quantified.

31. **(Withdrawn)** The method of Claim 30, wherein the quantification of mRNA of DNA-repair genes is used to test the sensitivity of DNA-repair genes to radiation.

32. **(Withdrawn)** The method of Claim 1, wherein the mRNA of allergen response genes is quantified.

33. **(Withdrawn)** The method of Claim 32, wherein the quantification of mRNA of allergen response genes is used to test allergen stimulation.

34. **(Currently amended)** The method of Claim 1, wherein the whole blood is exposed to donor cells prior to filtration, and wherein the mRNA of donor cell-mediated cytokines is quantified.

35. **(Currently amended)** The method of Claim 34, wherein the quantification of a higher than normal level of the mRNA of donor cell-mediated cytokines is used to test indicative of transplant rejection.

36. **(Original)** The method of Claim 1, additionally comprising determining the quantity of target mRNA in the sample using spiked control RNA.

37. **(Original)** The method of Claim 1, additionally comprising application of specific antisense primers during said lysate transferring step.

38. **(Original)** The method of Claim 1, additionally comprising application of specific antisense primers during said mRNA quantification step.

39-72. **(Cancelled)**

73. **(Currently amended)** A method of determining a definite quantity of target mRNA in a blood sample comprising:

- (a) collecting whole blood;
- (b) administering an anticoagulant to the whole blood;
- (c) removing erythrocytes and blood components other than leukocytes from the whole blood to yield leukocytes;
- (d) lysing the leukocytes with a lysis buffer containing spiked control RNA to produce a lysate comprising mRNA and spiked control RNA;
- (e) transferring the lysate to an oligo(dT)-immobilized plate to capture the mRNA;
- (f) quantifying the sample mRNA and spiked control RNA;
- (g) determining the percent recovery of spiked control RNA by dividing the value of spiked control RNA determined in step (f) by the amount of spiked control RNA obtained in step (d); and
- (h) determining the definite quantity of mRNA by dividing the value of sample mRNA determined in step (f) by applying the percent recovery of spiked control RNA determined in step (g).

74. **(Original)** The method of Claim 73, wherein the spiked control RNA is not homologous to RNA present in the blood sample.

75. **(Original)** The method of Claim 73, wherein step (b) comprises filtration to yield leukocytes on a filter membrane.

76. **(Cancelled)**

77. **(Original)** The method of Claim 73, wherein heparin is administered to the whole blood prior to collection of leukocytes.

78. **(Currently amended)** The method of Claim 73, wherein the whole blood is frozen and subsequently thawed prior to filtration.

79. **(Original)** The method of Claim 75, wherein the filter membrane is attached to a multi-well filter plate.

80. **(Currently amended)** The method of Claim 79, wherein 10 to 4×10^{10} 1×10^{10} copies of spiked control RNA are applied to each ~~filter plate~~ filter plate.

81. **(Currently amended)** The method of Claim 79, wherein 4×10^5 to 4×10^{10} copies of spiked control RNA are applied to each ~~filter~~plate filter plate.
82. **(Original)** The method of Claim 75, wherein the filter membrane is a PBT fibrous membrane.
83. **(Original)** The method of Claim 75, wherein the leukocytes are captured on a plurality of filter membranes layered together.
84. **(Original)** The method of Claim 75, additionally comprising washing the leukocytes on the filter membrane with hypotonic buffer to further remove erythrocytes and other blood components.
85. **(Original)** The method of Claim 84, additionally comprising drying the filter membrane.
86. **(Original)** The method of Claim 85, wherein the filter membrane is washed with ethanol and subjected to vacuum aspiration until the filter membrane is dry.
87. **(Original)** The method of Claim 73, wherein the immobilized plate comprises a multi-well oligo(dT)-immobilized plate.
88. **(Original)** The method of Claim 73, wherein the transfer of lysate to the oligo(dT)-immobilized plate comprises centrifugation.
89. **(Withdrawn)** The method of Claim 73, wherein the transfer of lysate to the oligo(dT)-immobilized plate comprises vacuum aspiration.
90. **(Withdrawn)** The method of Claim 73, wherein the transfer of lysate to the oligo(dT)-immobilized plate comprises applying positive pressure.
91. **(Original)** The method of Claim 73, wherein the quantification of mRNA comprises cDNA synthesis of the specific mRNA and amplification of resulting cDNA.
92. **(Original)** The method of Claim 73, additionally comprising application of specific antisense primers during said lysate transferring step.
93. **(Original)** The method of Claim 73, additionally comprising application of specific antisense primers during said mRNA quantification step.
- 94-214. **(Cancelled)**
215. **(New)** A method of high throughput quantification of a specific mRNA, comprising the steps of:
- (a) collecting whole blood;

- (b) administering an anticoagulant to the whole blood;
- (c) removing erythrocytes and blood components other than leukocytes from the whole blood by filtration to yield leukocytes on a filter membrane;
- (d) lysing the leukocytes on said filter membrane with a lysis buffer comprising antisense primers specific to said specific mRNA to produce a lysate comprising mRNA comprising said specific mRNA with said antisense primers hybridized thereto;
- (e) transferring the lysate to an oligo(dT)-immobilized plate to capture the specific mRNA;
- (f) removing non-hybridized materials from said oligo(dT)-immobilized plate;
- (g) adding reverse transcriptase to said oligo(dT)-immobilized plate without addition of primers, thereby synthesizing cDNA formed by extension of both the immobilized oligo(dT) and the antisense primers,
wherein the cDNA formed by extension of oligo(dT) remains immobilized to said plate, and the cDNA formed by extension of the antisense primers is in solution; and
- (h) quantifying the specific mRNA from said cDNA solution.

216. (New) The method of Claim 215, wherein the cDNA formed by extension of the antisense primers goes into solution without heat denaturation.

217. (New) The method of Claim 215, wherein a plurality of different antisense primers for different specific mRNAs are present in the lysis buffer.

218. (New) The method of Claim 217, wherein each of said different mRNAs is amplified from the cDNA formed in step (g).

219. (New) The method of Claim 217, wherein the cDNA solution is removed from the plate and the plate with the immobilized cDNA is stored for future use.